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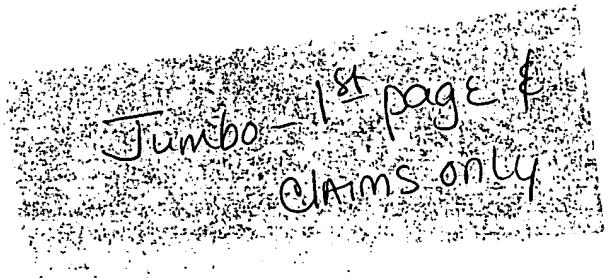
(54) Title: VIRAL VECTOR WITH BOVINE VIRAL DIARRHEA VIRUS (BVDV) ANTIGENS

(57) Abstract

This invention relates to the field of Bovine Viral Diarrhea Virus (BVDV), and vaccines for the treatment thereof. This invention describes the preparation of live, attenuated Bovine Herpesvirus type 1 (BHV-1) as a virus, vaccine and vector for expression of BVDV antigens. A BVDV cDNA clone containing sequences corresponding to glycoprotein gp53 is inserted into an inactivated BHV-1 virus.



Claims

1. A replicating nonpathogenic virus, for preventing disease caused by Bovine Viral Diarrhea Virus (BVDV), where said replicating nonpathogenic virus comprises:

a gene or gene combination taken from a BVDV virus, and said replicating nonpathogenic virus functionally expresses said gene or gene combination.

2. A virus of claim 1, where said replicating nonpathogenic virus is attenuated.

3. A virus of claim 2, where said replicating nonpathogenic virus is selected from attenuated Bovine Herpes Virus type 1 (BHV-1), attenuated adenoviruses, attenuated bovine mammillitis virus, attenuated bovine papillomavirus, or attenuated pseudorabies virus.

4. A virus of claim 2, where said replicating nonpathogenic virus is attenuated and contains and expresses any combination of the following genes: the genes that code for gp48, gp25, p14 capsid protein, p20 N-terminal protease and p125/p80 protein.

5. A virus of claim 3, where said replicating nonpathogenic virus is attenuated and contains and expresses any combination of the following genes: the genes that code for gp48, gp25, p14 capsid protein, p20 N-terminal protease and p125/p80 protein.

6. A virus of claim 2, where said attenuation is created by making the thymidine kinase (tk) gene nonfunctional.

7. A virus of claim 3, where said attenuation is created by making the thymidine kinase (tk) gene nonfunctional.

8. A virus of claim 4, where said attenuation is created by making the thymidine kinase (tk) gene nonfunctional.

9. A virus of claim 5, where said attenuation is created by making the thymidine kinase (tk) gene nonfunctional.

10. A virus of claim 9, where said replicating nonpathogenic virus is attenuated Bovine Herpes Virus type 1 (BHV-1).

11. A virus of claim 10, where said replicating nonpathogenic virus contains and expresses the gene that codes for gp53, a glycoprotein of the Bovine Viral Diarrhea Virus (BVDV).

12. A virus of claim 11, where a signal peptide is inserted preceeding the gene or gene combination that codes for gp53 in said Bovine Herpes Virus type 1 (BHV-1).

13. A virus of claim 12, where said gene that codes for gp53 is inserted into the inactivated thymidine kinase (tk) gene site.

14. A virus of claim 13, where the functionally expressing gene or gene combination, used to create the virus, comprises a recombined plasmid with intact viral DNA, said plasmid comprising:

a) a BHV-1 genomic DNA fragment containing the thymidine kinase (tk) gene and having a deletion to the thymidine kinase (tk) gene,

b) a promoter/polyadenylation signal inserted in the thymidine kinase (tk) gene deletion,

c) a signal peptide gene sequence preceding a gp53 gene or gene combination all of which is inserted between the promoter and the polyadenylation signal.

15. A virus of claim 14, where said signal peptide gene sequence is taken from any well characterized signal peptide sequences such as any of the thirty-nine examples of well characterized signal peptide sequences found in Perlman, D., et al., *J. Mol . Biol.* Vol. 167 pp. 391-409 (1983).

16. A virus of claim 15, where said signal peptide gene sequence is taken from Psuedorabies Virus gIII gene (PRV) and/or Bovine Growth Hormone (BGH).

17. A virus of claim 16 where the plasmid is selected from the following plasmids,

a) pBHVtkex-1::BGH/p53;

b) pBHVtkex-1::gIII/p53;

c) pBHVtkex-3::BGH/p53; or

d) pBHVtkex-3::gIII/p53.

18. A virus of claim 17, where said virus that produces the product of a functionally expressing gene or gene combination is selected from one of the following viruses,

T11-3, T11-6, or T11-8.

19. A virus of claim 18, where said gene or gene combination is T11-6.

20. A virus of claim 11, where said gene that codes for gp53 is inserted into the inactivated thymidine kinase (tk) gene site.

21. A virus of claim 20, where the functionally expressing gene or gene combination, used to create the virus, comprises a recombined plasmid with intact viral DNA, said plasmid comprising:

a) a BHV-1 genomic DNA fragment containing the thymidine kinase (tk) gene and having a deletion to the thymidine kinase (tk) gene,

b) a promoter/polyadenylation signal inserted in the thymidine kinase (tk) gene deletion,

c) a gp53 gene or gene combination inserted between the promoter and the polyadenylation signal.

22. A virus of claim 21, where said plasmid is made from a plasmid having the characteristics of plasmid pHAS4.

23. A virus of claim 22, where said plasmid is pBHVtkex-3::p53.

24. A virus of claim 23, where said virus is selected from one of the following viruses,

T2-3#3 or T2-2#5.

25. A vaccine for preventing disease caused by Bovine Viral Diarrhea Virus (BDVD) comprising a pharmaceutically effective amount of a virus of claim 1 and a carrier.

26. A vaccine as claimed in claim 25, for preventing disease caused by Bovine Viral Diarrhea Virus (BDVD) comprising a pharmaceutically effective amount of a virus of claim 1 and a carrier, said carrier comprising any physiological buffered medium, i.e. about pH 7.0 to 7.4 containing from about 2.5 to 15% serum which does not contain antibodies to BHV.

27. A method of immunizing an animal against infectious disease caused by Bovine Viral Diarrhea Virus (BDVD) comprising administering to an animal a pharmaceutically effective amount of a virus of claim 1.

28. A process of preparing a virus of claim 1 comprising:

a) isolation of a functionally expressing gene or gene combination that causes BVDV,

b) inserting said gene or gene combination into a replicating nonpathogenic virus,

c) selecting a live virus that functionally expresses the product of said gene or gene combination.

29. A method of preparing a virus of claim 11 where the functionally expressing gene or gene combination, used to create the virus, is produced by a process comprising the recombination of a plasmid with intact viral DNA, said plasmid comprising:

a) a BHV-1 genomic DNA fragment containing the thymidine kinase (tk) gene and having a deletion to the thymidine kinase (tk) gene,

b) inserting into the thymidine kinase (tk) gene deletion of said plasmid a promoter/polyadenylation signal,

c) inserting a gp53 gene or gene combination between the promoter and the polyadenylation signal,

d) transfecting cells with said plasmid to produce a recombinate virus containing said functional gene or gene combination inserted into a live virus that does not cause immunosuppression in the usual host and expressing said functional gene or gene combination.

30. A method of preparing a virus of claim 12 where the functionally expressing gene or gene combination, used to create the virus, is produced by a process

comprising the recombination of a plasmid with intact viral DNA, said plasmid comprising:

a) a BHV-1 genomic DNA fragment containing the thymidine kinase (tk) gene and having a deletion to the thymidine kinase (tk) gene,

b) inserting into the thymidine kinase (tk) gene deletion of said plasmid a promoter/polyadenylation signal,

c) inserting a gp53 gene or gene combination preceded by a signal peptide gene sequence between the promoter and the polyadenylation signal,

d) transfecting cells with said plasmid to produce a recombinate virus containing said functional gene or gene combination inserted into a live virus that does not cause immunosuppression in the usual host and expressing said functional gene or gene combination.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/U_ 94/12198

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/86 C07K14/18 C12N7/01 A61K39/12 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,94 00586 (RHONE-MERIEUX) 6 January 1994 see claim 11 ---	1-3,25, 27,28
Y	VIROLOGY, vol.190, no.2, 1992 pages 666 - 673 BELLO, L. ET AL. 'Bovine herpesvirus 1 as a live virus vector for expression of foreign genes' see the whole document --- -/--	1-3,6,7, 25,27-30

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 May 1995	07.06.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Chambonnet, F

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 94/12198

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	VIROLOGY, vol.190, no.2, 1992 pages 763 - 772 PATON, D.J. ET AL. 'Epitope mapping of the gp53 envelope protein of bovine viral diarrhea virus' see the whole document	1-3,6,7; 25,27-30
P,A	VIRUS RESEARCH, vol.34, no.2, 1994 pages 178 - 186 YU, M. ET AL. 'High level expression of the envelope glycoprotein (GP53) of bovine viral diarrhoea virus (singer) and its potential use as diagnostic reagent' see the whole document	1
A	EP,A,0 464 010 (STATENS VETERINÄRMEDICINSKA ANSTALT) 2 January 1992 see the whole document	1
A	WO,A,90 01337 (INSTITUTE FOR ANIMAL HEALTH LIMITED) 22 February 1990 see the whole document	1
A	EP,A,0 119 025 (BAYLOR COLLEGE OF MEDECINE. NOVAGENE LTD) 19 September 1984 see the whole document	1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/12198

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim 27 is directed to a method of treatment of the animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 94/12198

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9400586	06-01-94	FR-A-	2693472	14-01-94
		AU-B-	4334193	24-01-94
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		CA-A-	1319634	29-06-93
		EP-A-	0427767	22-05-91
		GB-A-	2239799	17-07-91
		JP-T-	4500069	09-01-92
EP-A-0119025	19-09-84	CA-A-	1237668	07-06-88
		US-A-	4569840	11-02-86